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DOCUMENT-IDENTIFIER: US 6210919 B1
TITLE: Genetic sequences and proteins related to alzheimer's disease

US PATENT NO. (1):
6210919

Brief Summary Text (9):
U.S. Pat. No. 5,297,562, describes detection of Alzheimer's Disease having two or more copies of chromosome 21. Treatment involves methods for reducing the proliferation of chromosome 21 trisomy. Canadian Patent application 2054302, describes monoclonal antibodies which recognize human brain cell nucleus protein encoded by chromosome 21 and are used to detect changes or expression due to Alzheimer's Disease or Down's Syndrome. The monoclonal antibody is specific to a protein encoded by human chromosome 21 and is linked to large pyramidal cells of human brain tissue.

Brief Summary Text (10):
By extensive effort and a unique approach to investigating the AD3 region of chromosome 14q, the Alzheimer's related membrane protein (ARMP) gene has been isolated, cloned and sequenced from within the AD3 region on chromosome 14q24.3. In addition, direct sequencing of RT-PCR products spanning this 3.0 kb cDNA transcript isolated from affected members of at least eight large pedigrees linked to chromosome 14, has led to the discovery of missense mutations in each of these different pedigrees. These mutations are absent in normal chromosomes. It has now been established that the ARMP gene is causative of familial Alzheimer's Disease type AD3. In realizing this link, it is understood that mutations in this gene can be associated with other cognitive, intellectual, or psychological diseases such as cerebral hemorrhage, schizophrenia, depression, mental retardation and epilepsy. These phenotypes are present in these AD families and these phenotypes have been seen in mutations of the APP protein gene. The Amyloid Precursor Protein (APP) gene is also associated with inherited Alzheimer's Disease. The identification of both normal and mutant forms of the ARMP gene and gene products has allowed for the development of screening and diagnostic tests for ARMP utilizing nucleic acid probes and antibodies to the gene product. Through interaction with the defective gene product and the pathway in which this gene product is involved, gene therapy, manipulation and delivery are now made possible.

Brief Summary Text (17):
In accordance with another aspect of the invention, are polyclonal antibodies raised to specific predicted sequences of the ARMP protein. Polypeptides of at least six amino acid residues are provided. The polypeptides of six or greater amino acid residues may define antigenic epitopes of the ARMP. Monoclonal antibodies having suitably specific binding affinity for the antigenic regions of the ARMP are prepared by use of corresponding hybridoma cell lines. In addition, other polyclonal antibodies may be prepared by inoculation of animals with suitable peptides or holoprotein which add suitable specific binding affinities for antigenic regions of the ARMP.

Brief Summary Text (27):
In accordance with another aspect of the invention an immuno therapy for treating a patient having Alzheimer's Disease comprises treating the patient with antibodies specific to the mutant ARMP to reduce biological levels or activity of the mutant ARMP in the patient. To facilitate such amino acid therapy, a vaccine composition may be provided for evoking an immune response in a patient of Alzheimer's Disease where the composition comprises a mutant ARMP and a pharmaceutically acceptable carrier with or without a suitable excipient. The antibodies developed specific to the mutant ARMP could be used to target appropriately encapsulated drugs/molecules, specific

cellular/tissue sites. Therapies utilizing specific ligands which bind to normal or wild type ARMP or either mutant or wild type and which augments normal function of ARMP in membranes and/or cells or inhibits the deleterious effect of the mutant protein are also made possible.

Detailed Description Text (25):
The mutations identified have been related to Alzheimer disease pathology. With the identification of sequencing of the gene and the gene product, probes and antibodies raised to the gene product can be used in a variety of hybridization and immunological assays to screen for and detect the presence of either a normal or mutated gene or gene product.

Detailed Description Text (26):
Patient therapy through removal or blocking of the mutant gene product, as well as supplementation with the normal gene product by amplification, by genetic and recombinant techniques or by immunotherapy can now be achieved. Correction or modification of the defective gene product by protein treatment immunotherapy (using antibodies to the defective protein) or knock-out of the mutated gene is now also possible. Familial Alzheimer's Disease could also be controlled by gene therapy in which the gene defect is corrected in situ or by the use of recombinant or other vehicles to deliver a DNA sequence capable of expressing the normal gene product, or a deliberately mutated version of the gene product whose effect counter balances the deleterious consequences of the disease mutation to the affected cells of the patient.

Detailed Description Text (68):
This amino acid similarity to ARMP suggest that the E5-1 homologue protein may be related to Alzheimer's Disease. Due to its structural similarity with the ARMP protein, the E5-1 homologue protein ay be used for the development of probes, peptides, or antibodies to various peptides which may recognize both the E5-1 and the ARMP gene and gene product, respectively. As a protein homologue for ARMP, the E5-1 homologue protein may be used as a replacement for a defective ARMP gene product. It may also be used to elucidate functions of the ARMP gene in tissue culture. A plasmid including this nucleic acid was deposited with the ATCC under the terms of the Budapest Treaty on Jun. 28, 1995 and has been assigned ATCC accession number 97214.

Detailed Description Text (70):
The ARMP protein is a member of a novel class of transmembrane proteins which share substantial amino acid homology. The homology is sufficient that certain nucleotide probes and antibodies raised against one can identify other members of this gene family. The major difference between members of this family reside in the amino acid and nucleotide sequence homologous to the hydrophilic acid loop domain between putative transmembrane 6 and transmembrane 7 domains of the ARMP gene and gene product. This region is alternatively spliced in some non-neural tissues, and is also the site of several pathogenic disease-causing mutations in the ARMP gene. The variable splicing of this hydrophilic loop, the presence of a high-density of pathogenic mutations within this loop, and the fact that the amino acid sequences of the loop differs between members of the gene family suggest that this loop is an important functional domain of the protein and may confer some specificity to the physiologic and pathogenic interactions which the ARMP gene product undergoes because the N-terminal hydrophilic domain shares the same acidic charge and same orientation with respect to the membrane, it is very likely that these two domains share functionality either in a coordinated (together) or independent fashion (eg. different ligands or functional properties). As a result everything said about the hydrophilic loop shall apply also to the N-terminal hydrophilic domain.

Detailed Description Text (91):
The ARMP gene and gene products will be useful for diagnosis of Alzheimer's disease, presenile and senile dementias, psychiatric diseases such as schizophrenia, depression, etc., and neurologic disease such as stroke and cerebral hemorrhage--all of which are seen to a greater or lesser extent in symptomatic subjects bearing mutations in the ARMP gene or in the APP gene. Diagnosis of inherited cases of these diseases can be accomplished by analysis of the nucleotide sequence (including genomic and cDNA sequences included in this patent). Diagnosis can also be achieved by monitoring alterations in the electrophoretic mobility and by the reaction with specific antibodies to mutant or wild-type ARMP gene products, and by functional assays demonstrating altered function of the ARMP gene product. In addition, the ARMP gene and ARMP gene products can be used to search for inherited anomalies in the gene and/or its products (as well as those of the homologous gene) and can also be used for diagnosis in the same way as they can be used for diagnosis of non-genetic cases.

Detailed Description Text (92):
Diagnosis of non-inherited cases can be made by observation of alterations in the ARMP transcription, translation, and post-translational modification and processing as well as alterations in the intracellular and extracellular trafficking of ARMP gene products in the brain and peripheral cells. Such changes will include alterations in the amount of ARMP messenger RNA and/or protein, alteration in phosphorylation state, abnormal intercellular location/distribution, abnormal extracellular distribution, etc. Such assays will include: Northern Blots (with ARMP-specific and ARMP-non-specific nucleotide probes which also cross-react with other members of the gene family), and Western blots and enzyme-linked immunosorbent assays (ELISA) (with antibodies raised specifically to ARMP; to various functional domains of ARMP; to other members of the homologous gene family; and to various post-translational modification states including glycosylated and phosphorylated isoforms). Theses assays can be performed on peripheral tissues (eg. blood cells, plasma, cultured or other fibroblast tissues, etc.) as well as on biopsies of CNS tissues obtained antimortem or postmortem, and upon cerebrospinal fluid. Such assays might also include in-situ hybridization and immunohistochemistry (to localized messenger RNA and protein to specific subcellular compartments and/or within neuropathological structures associated with these disease such as neurofibrillary tangles and amyloid plaques).

Detailed Description Text (105):
As an embodiment of the present invention, AMRP protein may be expressed using eukaryotic and prokaryotic expression systems. Eukaryotic expression systems can be used for many studies of the ARMP gene and gene product including determination of proper expression and port-translational modifications for full biological activity, identifying regulatory elements located in the 5' region of the large amounts of the normal and mutant protein for isolation and purification, to use cells expressing the ARMP protein as a functional assay system for antibodies generated against the protein or to test effectiveness of pharmacological agents, or as a component of a signal transduction system, to study the function of the normal complete protein, specific portions of the protein, or of naturally occurring and artificially produced mutant proteins.

Detailed Description Text (109):
These prokaryotic expression systems allow the holo-protein or various important functional domains of the protein to be recovered as fusion proteins and then used for binding studies, structural studies, functional studies, and for the generation of appropriated antibodies.

Detailed Description Text (111):
The DNA sequence can be manipulated in studies to understand the expression of the gene and its product, to achieve production of large quantities of the protein for functional analysis, for antibody production, and for patient therapy. The changes in the sequence may or may not alter the expression pattern in terms of relative quantities, tissue-specificity and functional properties. Partial or full-length DNA sequences which encode for the ARMP protein, modified or unmodified, may be ligated to bacterial expression vectors. E Coli can be used using a variety of expression vector system eg. the T7 RNA polymerase/promoter system using two plasmids or by labeling of plasmid-encoded proteins, or by expression by infection with M13 Phage mGPI-2. E. Coli vectors can also be used with Phage lamba regulatory sequence, by fusion and by glutathione-S-transferase fusion proteins, etc., all of which together with many other prokaryotic expression systems are widely available commercially.

Detailed Description Text (121):
Antibodies to Detect ARMP

Detailed Description Text (122):
Antibodies to epitopes with the ARMP protein can be raised to provide information on the characteristics of the proteins. Generation of antibodies would enable the visualization of the protein in cells and tissues using Western blotting. In this technique, proteins are run on polyacrylamide gel and then transferred onto nitrocellulose membranes. These membranes are then incubated in the presence of the antibody (primary), then following washing are incubated to a secondary antibody which is used for detection of the protein-primary antibody complex. Following repeated washing, the entire complex is visualized using colourimetric or chemiluminescent methods.

Detailed Description Text (123):

Antibodies to the ARMP protein also allow for the use of immunocytochemistry and immunofluorescence techniques in which the proteins can be visualized directly in cells and tissues. This is most helpful in order to establish the subcellular location of the protein and the tissue specificity of the protein.

Detailed Description Text (124):
In order to prepare polyclonal antibodies, fusion proteins containing defined portions or all of the ARMP protein can be synthesized in bacteria by expression of corresponding DNA sequences in a suitable cloning vehicle. The protein can then be purified, coupled to a carrier protein and mixed with Freund's adjuvant (to help stimulate the antigenic response by the rabbits) and injected into rabbits or other laboratory animals. Alternatively, protein can be isolated from cultured cells expressing the protein. Following booster injections at bi-weekly intervals, the rabbits or other laboratory animals are then bled and the sera isolated. The sera can be used directly or purified prior to use, by various methods including affinity chromatography, Protein A-Sepharose, Antigen Sepharose, Anti-mouse-Ig-Sepharose. The sera can then be used to probe protein extracts run on a polyacrylamide gel to identify the ARMP protein. Alternatively, synthetic peptides can be made to the antigenic portions of the protein and used to innoculate the animals.

Detailed Description Text (125):
To produce monoclonal ARMP antibodies, cells actively expressing the protein are cultured or isolated from tissues and the cell membranes isolated. The membranes, extracts, or recombinant protein extracts, containing the ARMP protein, are injected in Freund's adjuvant into mice. After being injected 9 times over a three week period, the mice spleens are removed and resuspended in phosphate buffered saline (PSB). The spleen cells serve as a source of lymphocytes, some of which are producing antibody of the appropriate specificity. These are then fused with a permanently growing myeloma partner cell, and the products of the fusion are plated into a number of tissue culture wells in the presence of a selective agent such as HAT. The wells are then screened to identify those containing cells making useful antibody by ELISA. These are then freshly plated. After a period of growth, these wells are again screened to identify antibody-producing cells. Several cloning procedures are carried out until over 90% of the wells contain single clones which are positive for antibody production. From this procedure a stable line of clones is established which produce the antibody. The monoclonal antibody can then be purified by affinity chromatography using Protein A Sepharose, ion-exchange chromatography, as well as variations and combinations of these techniques.

Detailed Description Text (128):
Antibodies may also be used coupled to compounds for diagnostic and/or therapeutic uses such as radionuclides for imaging and therapy and liposomes for the targeting of compounds to a specific tissue location.

Detailed Description Text (132):
The A.beta. peptide derivatives of APP are neurotoxic (Selkoe et al, 1994). APP is metabolized by passages through the Golgi network and then to secretory pathways via clathrin-coated vesicles with subsequent passage to the plasma membrane where the mature APP is cleaved by .alpha.-secretase to a soluble fraction (Protease Nexin II) plus a non-amyloidogenic C-terminal peptide (Selkoe et al. 1995, Gandy et al. 1993). Alternatively, mature APP can be directed to the endosome-lysosome pathway where it undergoes beta and gamma secretase cleavage to produce the A.beta. peptides. The phosphorylation state of the cell determines the relative balance of .alpha.-secretase (non-amyloidogenic) or A.beta. pathways (amyloidogenic pathway) (Gandy et al. 1993). The phosphorylation state of the cell can be modified pharmacologically by phorbol esters, muscarinic agonists and other agents, and appears to be mediated by cytosolic factors (especially protein kinase C) acting upon an integral membrane protein in the Golgi network, which we propose to be the ARMP, and members of the homologous family (all of which carry several phosphorylation consensus sequences for protein kinase C). Mutations in the ARMP gene will cause alterations in the structure and function of the ARMP gene product leading to defective interactions with regulatory elements (eg. protein kinase C) or with APP, thereby promoting APP to be directed to the amyloidogenic endosome-lysosome pathway. Environmental factors (viruses, toxins, and aging etc) may also have similar effects on ARMP. To treat Alzheimer's disease, the phosphorylation state of ARMP can be altered by chemical and biochemical agents (eg. drugs, peptides and other compounds) which alter the activity of protein kinase C and other protein kinases, or which alter the activity of protein phosphatases, or which modify the availability of ARMP to be postranslationally modified. The interactions between kinases and phosphatases with the ARMP gene products (and the products of its

homologues), and the interactions of the ARMP gene products with other proteins involved in the trafficking of APP within the Golgi network can be modulated to decrease trafficking of Golgi vesicles to the endosome-lysosome pathway thereby promoting A.beta. peptide production. Such compounds will include: peptide analogues of APP, ARMP, and homologues of ARMP as well as other interacting proteins, lipids, sugars, and agents which promote differential glycosylation of ARMP and its homologues; agents which alter the biologic half-life of messenger RNA or protein of ARMP and homologues including antibodies and antisense oligonucleotides; and agents which act upon ARMP transcription.

Detailed Description Text (133):
The effect of these agents in cell lines and whole animals can be monitored by monitoring: transcription; translation; post-translational modification of ARMP (eg phosphorylation or glycosylation); and intracellular trafficking of ARMP and its homologues through various intracellular and extracellular compartments. Methods for these studies include Western and Northern blots; immunoprecipitation after metabolic labelling (pulse-chase) with radio-labelled methionine and ATP, and immunochistochemistry. The effect of these agents can also be monitored using studies which examine the relative binding affinities and relative amounts of ARMP gene products involved in interactions with protein kinase C and/or APP using either standard binding affinity assays or co-precipitation and Western blots using antibodies to protein kinase C, APP or ARMP and its homologues. The effect of these agents can also be monitored by assessing the production of A.beta. peptides by ELISA before and after exposure to the putative therapeutic agent (Huang et al. 1993). The effect can also be monitored by assessing the viability of cell lines after exposure to aluminum salts and to A.beta. peptides which are thought to be neurotoxic in Alzheimer's disease. Finally, the effect of these agents can be monitored by assessing the cognitive function of animals bearing: their normal genotype at APP or ARMP homologues; or bearing human APP transgenes (with or without mutations); or bearing human ARMP transgenes (with or without mutations); or a combination of all of these.

Detailed Description Text (135):
The ARMP gene product and especially the gene product for the E5-1 homologue have amino acid sequence homology to human ion channel proteins and receptors. For instance, the E5-1 homologue shows substantial homology to the human sodium channel .alpha.-subunit (E=0.18, P=0.16, identities=22-27% over two regions of at least 35 amino acid residues) using the BLASTP paradigm of Altschul et al. 1990. Other diseases (such as malignant hyperthermia and hyperkalemic periodic paralysis in humans and the neurodegenerative of mechanosensory neurons in C. elegans) arise through mutations in ion channels or receptor proteins. Mutation of the ARMP gene and/or mutations in homologues could affect similar functions and lead to Alzheimer's disease and other psychiatric and neurological diseases. Based upon this, a test for Alzheimer's disease can be produced to detect an abnormal receptor or an abnormal ion channel function related to abnormalities that are acquired or inherited in the ARMP gene and its product, or in one of the homologous genes and their products. This test can be accomplished either in vivo or in vitro by measurements of ion channel fluxes and/or transmembrane voltage or current fluxes using patch clamp, voltage clamp and fluorescent dyes sensitive to intracellular calcium or transmembrane voltage. Defective ion channel or receptor function can also be assayed by measurements of activation of second messengers such as cyclic AMP, cGMP tyrosine kinases, phosphates, increases in intracellular Ca.sup.2+ levels, etc. Recombinantly made proteins may also be reconstructed in artificial membrane systems to study ion channel conductance. Therapies which affect Alzheimer's disease (due to acquired/inherited defects in the ARMP gene; due to defects in other pathways leading to this disease such as mutations in APP; and due to environmental agents) can be tested by analysis of their ability to modify an abnormal ion channel or receptor function induced by mutation in the ARMP gene or in one of its homologues. Therapies could also be tested by their ability to modify the normal function of an ion channel or receptor capacity of the ARMP gene products and its homologues. Such assays can be performed on cultured cells expressing endogenous normal or mutant ARMP genes/gene products (or its homologues). Such studies can be performed in addition on cells transfected with vectors capable of expressing ARMP, parts of the ARMP gene and gene product, mutant ARMP, or one its homologues (in normal or mutant form). Therapies for Alzheimer's disease can be devised to modify an abnormal ion channel or receptor function of the ARMP gene or its homologue. Such therapies can be conventional drugs, peptides, sugars, or lipids, as well as antibodies or other ligands which affect the properties of the ARMP gene product. Such therapies can also be performed by direct replacement of the ARMP gene and/or its homologue by gene therapy. In the case of an ion channel, the gene therapy could be performed using either mini-genes (cDNA plus a promoter) or genomic constructs bearing genomic DNA sequences for parts or all of the

ARMP gene. Mutant ARMP or homologous gene sequences might also be used to counter the effect of the inherited or acquired abnormalities of the ARMP gene as has recently been done for replacement of the mec 4 and deg 1 in C. elegans (Huang and Chalfie, 1994). The therapy might also be directed at augmenting the receptor or ion channel function of the homologous genes such as the E5-1 homologue, in order that it may potentially take over the functions of the ARMP gene rendered defective by acquired or inherited defects. Therapy using antisense oligonucleotides to block the expression of the mutant ARMP gene, coordinated with gene replacement with normal ARMP or a homologous gene can also be applied using standard techniques of either gene therapy or protein replacement therapy.

Detailed Description Text (146):
Immunotherapy is also possible for Alzheimer's Disease. Antibodies can be raised to a mutant ARMP protein (or portion thereof) and then be administered to bind or block the mutant protein and its deliterious effects. Simultaneously, expression of the normal protein product could be encouraged. Administration could be in the form of a one time immunogenic preparation or vaccine immunization. An immunogenic composition may be prepared as injectables, as liquid solutions or emulsions. The ARMP protein may be mixed with pharmaceutically acceptable excipients compatible with the protein. Such excipients may include water, saline, dextrose, glycerol, ethanol and combinations thereof. The immunogenic composition and vaccine may further contain auxiliary substances such as emulsifying agents or adjuvants to enhance effectiveness. Immunogenic compositions and vaccines may be administered parenterally by injection subcutaneously or intramuscularly.

Detailed Description Text (175):
These prokaryotic expression systems allow the holo-protein or various important functional domains of the protein to be recovered as fusion proteins and then used for binding studies, structural studies, functional studies, and for the generation of appropriate antibodies.

Detailed Description Text (183):
Polyclonal Antibody Production

Detailed Description Text (184):
Peptide antigens were synthesized by solid-phase techniques and purified by reverse phase high pressure liquid chromatography. Peptides were covalently linked to keyhole limpet hematoxylin (KLH) via disulfide linkages that were made possible by the addition of a cystein residue at the peptide C-terminus. This additional residue does not appear normally in the protein sequence and was included only to facilitate linkage to the KLH molecule. A total of three rabbits were immunized with peptide-KLH complexes for each peptide antigen and were then subsequently given booster injections at seven day intervals. Antisera were collected for each peptide and pooled and IgG precipitated with ammonium sulfate. Antibodies were then affinity purified with Sulfo-link agarose (Pierce) coupled with the appropriate peptide. This final purification is required to remove non-specific interactions of other antibodies present in either the pre- or post-immune system.

Detailed Description Text (185):
The specific sequences to which we have raised antibodies are;

Detailed Description Text (186):
Polyclonal antibody 1: NDNRERQDHNDRRSL (C)--residues 30-45 SEQ ID NO: 164

Detailed Description Text (187):
Polyclonal antibody 2: KDGQLIYTPFTEDTE (C)--residues 109-120 SEQ ID NO: 165

Detailed Description Text (188):
Polyclonal antibody 3: EAQRRVSKNSKYNAE (C)--residues 304-319 SEQ ID NO: 166

Detailed Description Text (189):
Polyclonal antibody 4: SHLGPHRSTPESRAA (C)--residues 346-360 SEQ ID NO: 167

Detailed Description Text (190):
The non-native cysteine residue is indicated at the C-terminal by (C). These sequences are contained within various predicted domains of the protein. For example, antibodies 1, 3, and 4 are located in potentially functional domains that are exposed to the aqueous media and may be involved in binding to other proteins critical for the development of the disease phenotype. Antibody 2 corresponds to a short linking region

situated between the predicated first and second transmembrane helices.